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MAIZE PLANT CONTRIBUTIONS TO ROOT ZONE AVAILABLE CARBON AND MICROBIAL TRANSFORMATIONS OF NITROGEN

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Summary—Root-derived C influences soil microbial activities that regulate N transformations and cycling in soil. The change in ^{13}C abundance of soil microbial biomass was used to quantify contributions from maize (*Zea mays* L.), a C_4 plant, to root zone-available C during growth in soil with a long history of C_3 vegetation. Effects of root-derived available C on microbial transformations of N were also evaluated using a $^{15}NH_4^{15}NO_3$ fertilizer tracer. Root-released C (microbial respired C_4-C + soil residue C_4-C) accounted for 12% (210 kg C ha^{-1}) of measured C fixed by maize at 4 wk and 5% at maturity when root-released C totaled 1135 kg C ha^{-1}. Of the C_4-C remaining in soil, only 18–23% was found in microbial biomass, indicating either a rapid turnover rate of biomass or a lower availability of C_4 substrates. Average daily production of root-derived available C was greatest during 4–8 wk maize growth (7 kg C ha^{-1} d^{-1}) when 4–11% of the soil microbial biomass came from this C source. At maize maturity, 15% of the microbial biomass (161 kg C ha^{-1}) came from root-derived available C, which totaled 402 kg ha^{-1}. Of the ^{15}N remaining in bare and cropped soils, averages of 23 and 16% (10 and 2 kg N ha^{-1}) were found in microbial biomass, and 64 and 2% (28 and 0.2 kg N ha^{-1}) were in inorganic ^{15}N form, leaving 13 and 82% (6 and 10 kg N ha^{-1}) as non-biomass organic N, respectively; this suggests that N cycling through microbial biomass was enhanced by root-derived C. Denitrification and N_2O losses from planted soils were low (1–136 g N ha^{-1} d^{-1}) when soil water-filled pore space (WFPS) was < 50%, but increased to 0.02–3.4 kg N ha^{-1} d^{-1} when soils were wetted to 85–95% WFPS when N_2 comprised 70–99% of denitrification products. The maximum denitrification rate was 1.5 times greater, and the cumulative denitrification losses 77% greater during early growth stages in planted soil as compared to bare soil when adequate NO_3^--N (> 2–3 mg kg^{-1}) was present in the soil. The presence of maize plants increased denitrification losses from soil by 19 to 57% (average of 29%) during early growth stages when the release of root-derived C was greatest. Published by Elsevier Science Ltd

INTRODUCTION

The balance of microbial mineralization-immobilization processes and microbial denitrification of NO_3^- are major factors determining the availability of soil N to crop plants during growth. The availability of C sources in soil is a major factor regulating the proliferation, death, and activity of the dominant heterotrophic soil microflora, and resultant N transformations such as mineralization-immobilization and microbial denitrification of NO_3^- (Ryden and Rolston, 1983; McCarty and Bremner, 1992). A clear understanding of available soil C supply during the growing season is essential to understanding how the supply of available N can be best managed in alternative systems that maximize N fertilizer use efficiency and minimize the potential for leaching of NO_3^--N into ground water or gaseous N losses to the atmosphere.

Part of the photosynthetically fixed C is translocated from the above-ground parts of the plant to the roots and subsequently into the surrounding soil as root respiration and root-derived material in the form of root exudates, mucilage, and sloughed cells and tissues. Total input of C to the soil by agricultural crop plants during the growing season represents 15–33% of the C assimilated by plants, which can amount to 900–3000 kg C ha^{-1} (Warembourg and Paul, 1973; Sauerbeck and Johnen, 1976; Johnen and Sauerbeck, 1977; Lucas *et al.*, 1977; Martin and Puckridge, 1982; Haller and Stolp, 1985). A zone of high microbial activity defined as the rhizosphere is created in the vicinity of growing roots by this input of organic material, which constitutes the main and immediately available source of substrate for the rhizosphere microflora (Barber and Martin, 1976; Biondini *et al.*, 1988; Johansson, 1992; Martin and Merckx, 1992). There is a substantial body of information on the amounts of root-derived C added to soil in highly

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disturbed soil–plant systems. However, little information is available on the contributions of root-derived C to the root zone available C pools under conditions that better reflect the field, and how this readily available C affects the microbial transformations of soil N. This is mainly due to methodological difficulties in quantifying root-derived available C. The objectives of our study were to further develop a practical method, based on an initial study (Qian and Doran, 1996), for quantifying crop plant contributions to root zone soil available C during plant growth and to test the hypothesis that root-derived available C enhances microbial transformations of N in soil, specifically immobilization-mineralization and gaseous N losses via microbial denitrification.

MATERIALS AND METHODS

A greenhouse study using ^{13}C natural abundance and isotope ^{15}N techniques was conducted with maize, a C_4-plant ($\delta^{13}C = -12‰$) and an important economic crop, to quantify root-derived available C and to identify microbial transformations of N as related to the supply of root-derived available C. The latter was quantified using the approach described by Qian and Doran (1996). The soil was a Crete silt loam, which was cropped with smooth biomegrass (*Bromus inermis* L.), a C_3-plant ($\delta^{13}C = -27‰$), for 25 y. Soil NH_4^+- and NO_3^--N were 1.3 and 19 mg kg^{-1} and total C and N were 17.9 and 1.7 g kg^{-1}, respectively. Initial soil pH was 5.5, and soil was amended with CaO at a rate of 0.5 g kg^{-1} soil to bring the soil pH to 6.3 as determined by the Woodruff buffer method (Eckert, 1988). Soil was fertilized, based on soil testing results, by adding of 27.54 mg $^{15}NH_4$ $^{15}NO_3$ with 99.2 atom% ^{15}N and 101.8 mg $Ca(H_2PO_4)_2 \cdot H_2O$ kg^{-1}, soil.

Two sizes of polyvinyl chloride (PVC) cylinders, eight short ones (445 × 289 mm inner dia) for the first two harvests and eight tall ones (787 × 289 mm inner dia) for the last two harvests were used. The soil was packed in the cylinders to a bulk density of 1.16 g cm^{-3} with 102-mm height headspace and planted with maize (*Zea mays* L.) with three replicates and four harvest dates in a randomized complete block design. One control (without maize) was included for each harvest date. The four harvest dates were chosen evenly at 4, 8, 12 and 16 wk after emergence, which were approximately at growth stages of V_{10} (tenth leaf), V_T (tasseling), R_3 (milk) and R_5 (dent), respectively. Maize seeds (Pioneer Brand 3379 hybrid) were soaked in water for 48 h and then five seeds per soil column were planted at a depth of 2.5 cm. After an initial growth of 5 d, seedlings were thinned to one per column and the soil surface covered with a fiber glass disc to limit evaporation. The maize was grown for 4–16 wk in the greenhouse, which was equipped with additional light sources to ensure a quantum flux density in a range of 180–400 $\mu E\ m^{-2}s^{-1}$. The plants were grown during daylight for 14 h at daytime average temperatures of 27°C and during night for 10 h at 21°C, which represent average growth conditions for maize in this climatic region. Soil water content of all columns was adjusted to 60% (WFPS) by weighing every 10 d during early growth stages and every 4 d at later stages when water use by maize was greatest. A second application of 99.2 atom% $^{15}NH_4^{15}NO_3$, at a rate of 20 kg N ha^{-1}, was made in the irrigation water after the first harvest.

Sampling schedules for gas flux measurements were arranged as close to soil sampling as possible (24 d after irrigation and gas sampling, depending on soil drying speed) to enable best comparison with soil variables measured immediately before and after plant harvest. For each gas sampling period, the soil cylinders were closed with Plexiglas lids with openings for the plant stems, vent and water supply. The lids were sealed with silicon rubber, and the openings around the stems were sealed with a Duct Seal (Gardner Bender Co.). Soils were wetted to 85–95% WFPS, to create anaerobic conditions and enhance microbial denitrification, by adding measured amounts of distilled water to the soil surface at the top of each soil column. After wetting, samples for CO_2 and N gas analyses were taken from the cylinder headspace four times at 24, 48, 72 and 96 h (four consecutive days) to ensure that gas fluxes were not confounded by restricted gas diffusion in wet soils. Wetting treatments, gas sampling schedules and procedures were identical for control and planted soils. For each time of gas sampling, gases were sampled at 0 min as a blank and at 60 min after the headspace was sealed. For each sample, 20 ml of headspace gas was withdrawn in pre-evacuated glass sampling vials, immediately scaled with silicon rubber, and transported to the laboratory for analyses. At the end of each gas sampling period, CO_2 in the headspace was trapped by evacuation through a scrubber with 1 m NaOH for 3 min and analyzed for $\delta^{13}C$ by the procedure used for microbial biomass $\delta^{13}C$ analysis as described by Qian and Doran (1996).

The N_2O was analyzed using a Shimadzu (GC-14A) g.c. fitted with an e.c.d. and CO_2 using a Tracor (MT-220) g.c. with a t.c.d. Details concerning the analysis of N_2O and CO_2 are given by Aulakh *et al.* (1991) and Bronson *et al.* (1992). The N_2O and CO_2 fluxes were calculated from their concentrations in the headspace over 1 h on a mass $ha^{-1}\ d^{-1}$ basis. Gaseous ^{15}N was analyzed using an Europa Scientific tracermass spectrometer equipped with a triple collector system (A. Kessavalou, 1995, unpublished procedure) according to Mulvaney and Kurtz (1982), Siegel *et al.* (1982), Mulvaney and

Boast (1986), and Mosier and Klemedtsson (1994). The N gas evolved due to denitrification was calculated based on the total N_2 in the headspace and the fraction of total N_2 gas in the headspace attributable to denitrification, which is proportional to the mole fraction of ^{15}N in the soil NO_3^- Pool (Mosier and Klemedtsson, 1994, p. 1059). Gaseous ^{15}N flux was calculated from the total N_2 in the headspace and its excess ^{15}N atom%. According to the gas fluxes from different gas sampling periods, cumulative losses of denitrification, N_2O emission, and gaseous ^{15}N for the four growth periods were estimated for the control and planted soils, respectively. These estimates were made by linearly interpolating data points for each day according to the gas flux measurements and integrating the area under the points for each day over the corn growing period (0–16 wk) using Simpson's Rule (Bronson *et al.*, 1992; Hanson *et al.*, 1994; Qian *et al.*, 1997).

Maize plants were harvested and soil was sampled from three planted soil columns and the nonplanted control soil at 4, 8, 12 and 16 wk after emergence. At each harvest date, shoots were cut at the soil surface and oven-dried (65°C). A 'come-along' device and piston apparatus were used to push the column of soil out of the cylinder. The cylindrical soil segments were then cut longitudinally into 1/8 sections like a pie and two 1/8 sections each for root and soil analyses samples for planted soil, and two 1/8 sections for soil samples of control soil, were bagged and stored at 4–6°C for transport to the laboratory. Root samples were collected by washing away soil with gently flowing tap water through a 2-mm sieve, oven-dried at 65°C followed by, as with shoot samples, determination of dry matter, total C and N, ^{15}N and $\delta^{13}C$. Composite soil samples were taken, after removal of roots by hand and sieving (< 2 mm), and temporarily stored at 5°C until taking subsamples for analysis of soil organic C and $\delta^{13}C$, microbial biomass C and $\delta^{13}C$, total soil N and ^{15}N, soil inorganic N (NH_4^+ and NO_3^-) and ^{15}N ($^{15}NH_4^+$ and $^{15}NO_3^-$), and microbial biomass N and ^{15}N.

Total C, total N, and ^{15}N of plant and soil were determined using a Carlo Erba automatic C and N analyzer interfaced with a continuous-flow Europa Scientific tracermass spectrometer as described by Schepers *et al.* (1989). The $\delta^{13}C$ of plant and soil were analyzed using the same system (A. Kessavalou, 1995, unpublished procedure). Soil NH_4^+ and NO_3^- contents were determined from 1:10 soil–KCl (2 m) extracts by the Indophenol Blue and cadmium reduction techniques, respectively (Keeney and Nelson, 1982). Soil $^{15}NH_4^+$ and $^{15}NO_3^-$ were also measured on the above soil–KCl extracts using the diffusion method (Brooks *et al.*, 1989; Kelley *et al.*, 1991). Excess ^{15}N atom% of soil total and inorganic N were calculated by subtracting background ^{15}N values, which, in most cases was equal to 0.3663%.

Microbial biomass C was measured by the chloroform fumigation–incubation method (Jenkinson, 1988). Soil mineralizable C was determined by CO_2-C evolved from 10- to 20-d incubation of non-fumigated soil sample during measurement of microbial biomass. The amount of microbial biomass C from C_4 plants, referred to as biomass C_4-C, was calculated from the $\delta^{13}C$ change of microbial biomass. Root-derived available C was obtained from the biomass C_4-C divided by a substrate utilization efficiency of microorganisms for which we used an approximate value of 40% (Qian and Doran, 1996). The balance of 60% was microbial respired C_4-C. The detailed description of above measurements and calculations are given by Qian and Doran (1996). Microbial biomass N was determined using the chloroform fumigation–incubation method with a $K_N = 0.68$, the proportion of microbial N mineralized to NH_4^+ and NO_3 from fumigated samples (Shen *et al.*, 1984). The ^{15}N analysis of KCl extractable N from fumigated and non-fumigated soil samples was performed by the diffusion method as referred to above for $^{15}NH_4^+$. Microbial biomass excess ^{15}N was calculated from the difference of excess KCl extracted ^{15}N in fumigated and non-fumigated soil samples divided by the same K_N factor (0.68).

The data were analyzed by ANOVA using Abacus Concepts (1989) SuperANOVA. The two-way analysis of variance was performed for main and interaction effects using the general linear model procedures for randomized complete block design. Following the *F*-test in ANOVA, multiple comparison of means over four harvest times was contrasted using Fisher's Protected Least Significant Differences. A probability level of 0.05 was used to test the significance of statistical hypothesis. Standard deviation and error was also computed for the means.

RESULTS AND DISCUSSION

Distribution of net photosynthate in plant–soil system

Net photosynthate, as used here, refers to total photosynthetically fixed C accumulated in the plant system, excluding any C respired by shoots and roots. Root- and shoot-C increased five- to sixfold between 4 and 8 wk and only one to 1.5-fold for roots and 1.3- to 1.8-fold for shoots between 8 and 12, and 12 and 16 wk after emergence (Table 1). Below-ground C_4-C, comprising root C, microbial respired C_4-C, and soil residue C_4-C, accounted for 33 to 20% of total net photosynthate C as growth proceeded. Martens (1990) estimated that the amount of ^{14}C-photosynthate translocated below ground for maize grown in a greenhouse decreased from 41% of total photosynthate at 6 wk to 21% at

Table 1. Distributions of assimilated C in the plant–soil system, soil $\delta^{13}C$, and C_4-C input to soil during growth of maize in a Crete silt loam with a prior history of C_3 vegetation

Component	Growth period (weeks after emergence)			
	4	8	12	16
	Distribution of net photosynthate (kg ha^{-1})			
Shoot C	1160 (11)[a]	7445(407)	13072(486)	17375(845)
Root C	368 (27)	1926 (71)	2838 (141)	3077 (197)
Microbe-respired C_4-C	53 (11)	170 (15)	213 (28)	241 (40)
Soil residue C_4-C	157 (34)	586 (59)	753 (48)	894 (104)
Biomass C_4-C	35 (8)	113 (10)	142 (19)	161 (26)
Biom./soil C_4-C (%)	22.5	19.3	18.9	18.0
	Percentage of total C fixed			
Below-ground C_4-C	33.3	26.5	22.5	19.5
Root C	21.2	19.0	16.8	14.3
Root released C	12.1	7.5	5.7	5.2
	Percentage of below-ground C_4-C			
Root C	63.7	71.8	74.6	73.0
Root-released C	36.3	28.2	25.4	27.0
Soil residue C_4-C	27.2	21.9	19.8	21.2
Biomass C_4-C	6.1	4.2	3.7	3.8
	Soil $\delta^{13}C$, difference, and C_4-C input to soil			
Control (‰)	−20.41 (0.10)	−20.12 (0.12)	−19.53 (0.12)	−19.25 (0.10)
Planted (‰)	−20.12 (0.16)	−19.25 (0.15)	−18.97 (0.14)	−18.61 (0.17)
$\delta^{13}C$ difference (‰)	0.29	0.87	0.56	0.64
C_4-C input (kg ha^{-1})	157 (34)	586 (59)	753 (48)	894 (104)

[a]Standard deviation (n = 3) given in parentheses.

15 wk. In our studies, root released C, comprising microbial respired C_4-C and soil residue C_4-C, accounted for 12% of total measured C fixed at early growth stage (4 wk) and 5% at maturity. The decrease of these percentages indicates a change in plant C allocation and distribution with growth stage. During early growth, more C was presumably allocated below ground for building the rapidly growing root system. Of total below ground C_4-C, root C accounted for 64–75% and the balance of 25–36% was present as rhizo-deposited C, mainly as soil organic and microbial residues. Davenport and Thomas (1988) reported that 10% of the ^{14}C assimilated by maize plants was translocated below ground. However, their estimate was probably low because microbial respired C_4-C was not included as translocated C. In other studies, fresh roots represented 85% and rhizo-deposition 15% of the total C input to soil at the harvest time of maize (Haller and Stolp, 1985; Hetier *et al.*, 1986).

In our study, a total of 1135 kg C ha^{-1} was excreted by plant roots by harvest time, which is within the range of previous estimates of total C inputs from roots of arable crops during the growing season (900–3000 kg C ha^{-1}). The proportion of the total C assimilated by these crop plants that is released into the soil ranges from 15 to 33% (Warembourg and Paul, 1973; Sauerbeck and Johnen, 1976; Keith *et al.*, 1986), and from 5 to 25% of net photosynthesis (Barber and Martin, 1976; Haller and Stolp, 1985; Milchunas *et al.*, 1985; Martin, 1987; Liljeroth *et al.*, 1990; Johansson, 1992).

Contribution of root-derived C to soil C pool

Soil $\delta^{13}C$ changed significantly ($P < 0.05$) with time and presence of maize (Table 1). The soil $\delta^{13}C$ of planted soils became less negative with time, presumably due to the addition of C_4 plant C. However, part of this effect may have resulted from preferential utilization by microorganisms of ^{12}C over ^{13}C substrates. Such isotopic fractionation would be supported by the data presented in Fig. 1, where the average $\delta^{13}C$ of respired C from the control soil (−21‰) was more negative than that of soil organic C (average −19.8‰, Table 1). Similar findings were reported by Mary *et al.* (1992) in a study of root material and glucose biodegradation in soil; they cautioned that additional research is needed to determine whether isotope discrimination

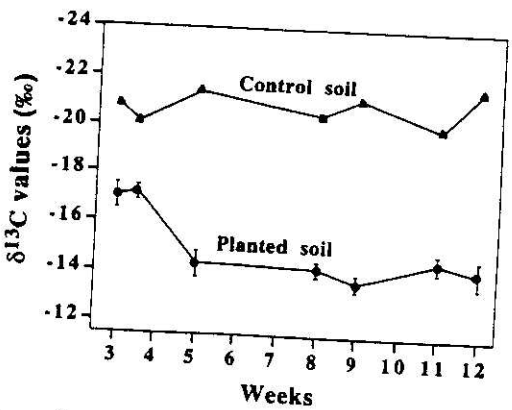

Fig. 1. $\delta^{13}C$ variations of respired soil C during growth of maize. Vertical bars show standard errors.

Table 2. Soil mineralizable C, Microbial biomass C, biomass $\delta^{13}C$, biomass C_4-C fraction, biomass C_4-C, and root-derived available C in the Crete silt loam (C_3 history)

Components	Growth period (weeks after emergence)			
	4	8	12	16
		Mineralizable C (kg ha^{-1} d^{-1}, 35 cm)		
Control	47.5 (1.5)[a]	34.1 (1.0)	38.0 (1.0)	34.2 (1.3)
Planted	54.6 (5.0)	53.9 (1.2)	57.3 (2.5)	51.1 (7.3)
		Microbial biomass C (kg ha^{-1}, 35 cm)		
Control	1010 (27)	890 (31)	855 (13)	851 (23)
Planted	904 (25)	1027 (29)	1061 (32)	1104 (39)
		Microbial biomass $\delta^{13}C$ ($\delta^{13}C_{MB}$, ‰)		
Control	−24.57 (0.18)	−24.56 (0.08)	−24.32 (0.19)	−24.50 (0.15)
Planted	−24.07 (0.10)	−23.18 (0.14)	−23.49 (0.11)	−23.58 (0.19)
$\delta^{13}C$ difference	0.50	1.38	0.83	0.92
		Microbial biomass C_4-C fraction (f, %)		
Planted	3.9 (1.1)	11.0 (1.1)	6.7 (1.4)	7.4 (1.6)
		Microbial biomass C_4-C (kg ha^{-1})		
Planted	35.3 (7.7)	113.2 (9.7)	142.1 (18.9)	160.9 (26.4)
		Root-derived available C (C_{av}, kg ha^{-1})		
Planted	88.3 (19.1)	283.0 (24.3)	355.4 (47.4)	402.3 (66.0)
		Average C_{av} production rate (kg ha^{-1} d^{-1})[b]		
Planted	3.2	7.0	2.6	1.7

[a]Standard deviation (n = 3) given in parentheses.
[b]Average C_{av} production rate = increase in C_{av} over 4 wk proceeding harvest/28.

is a general phenomenon in substrate decomposition in soil. The $\delta^{13}C$ of respired C from planted soil was much less negative than that of the organic C in the planted soil because root respiration and microbial respiration of root exudates, both with a $\delta^{13}C$ near −14‰, contributed 36–82% (average 65%) of total soil respiration, which was very similar to the range of 40–100% found by Robinson and Scrimgeour (1995). However, no attempt was made in these studies to separate the relative contributions between root respiration and microbial respiration using root-released C as a substrate.

More importantly, however, in addition to change with time due to microbial processing of C, the $\delta^{13}C$ of planted soil also changed with growth and, presumably, root exudation of maize. The difference in soil $\delta^{13}C$ values between control and planted soils was used to calculate C_4-C inputs to soil from maize as shown in Table 1. The difference in soil $\delta^{13}C$ values between planted and control soils for the third and fourth harvests were smaller than those of the second harvest because the effects of C_4-C inputs on the $\delta^{13}C$ signal were diluted by the larger soil volume (twice as much) for the last two harvests. However, the absolute amounts of C_4-C entering and remaining in the soil for the last two harvests were still greater than those of the first two harvests. The amount of C_4-C input increased dramatically (near fourfold) between the first and second harvests and then slowly but significantly increased between the second and last harvests. The C_4-C remaining in soil (soil residue C_4-C) accounted for 27–20% (average of 23%) of the net amount of photosynthate translocated below ground (Table 1).

Contribution of root-derived C to root zone available C

Soil mineralizable C, an index of root zone available C, in control and planted soils was fairly stable, except for a significant decrease from the first to second harvest in the non-planted control soil (Table 2). The mineralizable C of planted soil was significantly greater than that of the control soil at various harvest times, presumably the result of root exudates, fine root turnover, and enhanced microbial decomposition of soil organic C in planted soil. Soil microbial biomass of control soil was fairly constant, except for a significant decrease (12%) from the first to the second harvest which was correlated with soil mineralizable C that is essential to microbial biomass maintenance and growth. However, the microbial biomass of planted soil increased 14% from the first to second harvest and then tended to increase slightly over time (Table 2). The average microbial biomass of planted soil (1024 kg ha^{-1}) was significantly greater (14%) than that of the control soil (902 kg ha^{-1}) which was apparently associated with microbial use of root-derived C.

After the introduction of the C_4-plant maize to the C_3 soil, the $\delta^{13}C$ values of both soil organic C (Table 1) and microbial biomass C (Table 2) became less negative, i.e. the ^{13}C became enriched in these C pools due to the incorporation of C_4-C into the microbial biomass and soil C pools. The $\delta^{13}C$ values of microbial biomass C, however, changed more rapidly than those of soil organic C, indicating that microorganisms were active in using fresh C_4-C sources from living roots. Unlike the total soil C, the $\delta^{13}C$ of microbial biomass C in control soil did not change significantly with time, whereas the $\delta^{13}C$ of microbial biomass in planted

soil became less negative from the first to the second harvest and then changed little or became slightly more negative with time. These changes in the $\delta^{13}C$ of microbial biomass in planted soil apparently resulted from changes in root released C_4-C and turnover of microbial biomass with time. The difference in the $\delta^{13}C$ between the control and planted soil was assumed to result solely from incorporation of root-derived C_4-C into the microbial biomass and was used to calculate the amount of C_4-C incorporated into the biomass. Similar to the variation of soil $\delta^{13}C$, the difference of the $\delta^{13}C$ between control and planted soils of the third and fourth harvest was smaller than that of the second harvest because the dilution of the $\delta^{13}C$ signature of added root-derived C by C sources from the 70 cm soil cores, which were double those of 35 cm cores.

The C_4-C fraction (f), which represents the proportion of microbial biomass derived from C_4 root material, showed similar patterns to the difference of microbial biomass $\delta^{13}C$ between control and planted soils. It is apparent from Table 2, if expressed on the same soil volume basis, that only a small fraction of soil microbial biomass (4–15%) profits from the supply of organic compounds from roots, indicating either a low efficiency of substrate use or a greater turnover rate.

These results were similar to those of Merckx *et al.* (1987) and Van Veen *et al.* (1989) who found that 16–17% of the microbial biomass was ^{14}C-labeled in maize-cropped soil. The amount of C_4-C incorporated into microbial biomass and root-derived available C increased considerably (over threefold) from the first to second harvest and then increased slowly from the second to the last harvest (Table 2). The microbial biomass C_4-C accounted for 6 to 4% of net photosynthate translocated belowground (Table 1). Of the C_4-C remaining in soil (soil residue C_4-C), 18–23% was found in microbial biomass (Table 1), indicating a low 'apparent' efficiency of substrate use or greater turnover rate of the microbial biomass. This agreed with previous results that root-derived products were slowly incorporated by the soil microbial biomass to a maximum of 20% of the residual soil ^{14}C content after 6 weeks' growth of wheat (Merckx *et al.*, 1986). Merckx *et al.* (1987) and Van Veen *et al.* (1989) also found that the amount of ^{14}C in the microbial biomass represented about 20% of the total ^{14}C present in both wheat- and maize-cropped soil. More detailed investigations of the chemical nature of the 80% non-biomass components of root-derived residue C in soil, including the microbial respired part, should provide more about whether this large pool of C is related to metabolized products, processed by a small but highly active microbial biomass, or represents fresh unaltered root-derived materials.

Root-released total C, comprising soil residue C_4-C and microbial respired C, accounted for 36 to 27%, with an average of 29% of photosynthate translocated belowground from the first to last harvest (Table 1). The decrease of these percentages with time indicated a change in plant C allocation and distribution with growth stage. Root-derived available C increased with growth period, but the increase from the first to second harvest (threefold) was much greater than the later growth periods, which increased only 26% between the second and third, and 13% between the third and fourth harvests. However, the proportion of root-derived available C in the total C_4-C entering the soil (soil residue C_4-C Plus microbial respired C_4-C) decreased with growth period from 42 to 35%. This trend suggested that a relatively greater proportion of easily decomposable substrate was exuded from younger than from older, more mature plant roots.

^{15}N distribution in plant–soil system

Excess ^{15}N in plants increased steadily with plant growth (Table 3). The fertilizer utilization efficiencies, however, varied with different harvest times from 35 to 48% with an average of 44%. Slightly more than half of the N applied remained in the soil or was lost as gaseous N. Excess ^{15}N in the control soil, directly associated with ^{15}N application, increased up to the third harvest with increasing ^{15}N fertilization of 39.5, 59.3, and 98.8 kg N ha^{-1} to the entire column of 35- and 70-cm depths, respectively (Table 3), and decreased somewhat from the third to fourth harvest (which received the same amount of ^{15}N fertilizer) because of the greater opportunity for N gaseous loss from soil. Excess ^{15}N in planted soil decreased from the first to the second harvest because of the intensive uptake of N as a result of rapid maize growth, even though 50% more ^{15}N was applied after the first harvest (19.8 kg N ha^{-1}). In spite of increasing plant uptake of ^{15}N, the soil excess ^{15}N increased from the second to the third harvest because of greater total ^{15}N applied (98.8 kg ^{15}N ha^{-1}) in the deeper (70 cm) soil columns and greater accessibility of soil available N in a larger soil volume not yet occupied by roots. Similar to the control soil, the excess ^{15}N in the planted soil declined again from the third to the fourth harvest, because of apparently greater plant uptake of ^{15}N and greater opportunity for N gaseous loss from soil as discussed later. Total soil ^{15}N in the control soil accounted for 32–106 kg N ha^{-1} which represented 80–107% of ^{15}N applied to soil at different sampling dates, which left 0–20% of ^{15}N unaccounted for (Table 3). Soil ^{15}N plus plant uptake of ^{15}N in planted soil accounted for 26–73 kg N ha^{-1}, which represented 64–74% of ^{15}N applied to soil at different harvest times with 26–36% of the applied ^{15}N unaccounted for.

Table 3. Distribution of ^{15}N in plant–soil system at various growth periods

Fates of ^{15}N	Growth period (weeks after emergence)			
	4	8	12	16
		kg ^{15}N ha^{-1}		
^{15}N excess applied	39.5	59.3	98.8	98.8
		Control soil		
Soil ^{15}N	32.2 (0.4)[a]	47.3 (0.8)	105.8 (0.2)	88.4 (1.2)
Unaccounted	7.3	12	0.0	10.4
		Planted soil		
Plant uptake	13.7 (0.3)	28.7 (1.9)	44.2 (2.0)	47.5 (3.1)
Soil ^{15}N	12.2 (1.3)	9.4 (1.2)	28.6 (2.2)	19.6 (1.8)
Unaccounted	13.6	21.2	26.0	31.7
		Percentage of soil ^{15}N		
		Control soil		
Inorganic ^{15}N	70.8	51.6	65.6	68.2
Organic ^{15}N	29.2	48.4	34.4	31.8
Biomass ^{15}N	26.6	25.4	20.9	21.7
		Planted soil		
Inorganic ^{15}N	5.4	1.1	0.7	0.5
Organic ^{15}N	94.6	98.9	99.3	99.5
Biomass ^{15}N	10.7	20.6	12.8	18.9

[a]Standard deviation (n = 3) given in parentheses.

Immobilization and mineralization of N

Total microbial biomass N of the nonplanted control soil decreased significantly from 4- to 8- to 12-wk sampling times, and there was no change between 12- and 16-wk values (Table 4). Biomass was negatively correlated with NO_3^- concentration in the control soil ($r = -0.934$). The observation that soil NO_3^- concentration increased with decreases in soil microbial biomass N could indicate continuous mineralization of biomass N followed by rapid nitrification because very small amounts of NH_4^+ were detected in the soil (Table 5). The total microbial biomass N of planted soil did not change significantly throughout the entire growth period, and there was no difference in average total microbial biomass-N amounts between control and planted soils, whereas a great difference was found between control and planted soils in microbial biomass excess ^{15}N. The average of biomass excess ^{15}N in control soil was ninefold greater than that in planted soil (Table 4). Particularly, the microbial biomass excess ^{15}N concentrations at different growth stages were significantly greater ($P < 0.05$) in control soil (average 10.4 kg ha^{-1}) than those in planted soil (average 1.7 kg ha^{-1}) because of plant uptake of fertilizer ^{15}N (Table 3). The microbial biomass excess ^{15}N in both control and planted soils was also changing over growth periods, resulting from immobilization and mineralization of fertilizer ^{15}N. The microbial biomass excess ^{15}N in control and planted soils increased significantly from the first to the second harvest (36 and 50% increase for control and planted soil, respectively) and then tended to decline in the control soils but remained unchanged throughout the rest of the growth period in planted soil. Although the plant uptake of ^{15}N increased, the biomass ^{15}N in planted soil also increased and remained stable, indicating that the supply of root-released C met the C needs of the soil microbial biomass and enabled soil microorganisms to compete for soil available N with plants.

Table 4. Dynamics of soil microbial biomass N and ^{15}N in control and planted soils

Components	Growth period (weeks after emergence)			
	4	8	12	16
		Total microbial biomass N (kg ha^{-1}, 35 cm)[a]		
Control	140.4 (2.2)[b]	120.4 (3.9)	111.3 (7.2)	112.7 (2.1)
Planted	121.2 (12.9)	127.8 (5.1)	126.9 (15.5)	128.2 (11.1)
		Microbial biomass excess ^{15}N (kg ha^{-1}, 35 cm)[a]		
Control	8.58 (1.09)	12.00 (0.80)	11.03 (0.92)	9.60 (0.81)
Planted	1.30 (0.11)	1.94 (0.19)	1.83 (0.16)	1.85 (0.18)
		Percentage of total biomass N to soil N		
Control	2.12	1.88	1.73	1.76
Planted	1.89	2.05	1.96	1.95
		Percentage of biomass ^{15}N to soil ^{15}N		
Control	26.6	25.4	20.9	21.7
Planted	10.7	20.6	12.8	18.9

[a]All quantitative values for 12 and 16 wk should be multiplied by 2 to obtain values for the entire 70-cm columns.
[b]Standard deviation (n = 3) given in parentheses.

Table 5. Dynamics of soil inorganic N and ^{15}N control and planted soils

Components ($kg\ ha^{-1}$)	Growth period (weeks after emergence)			
	4	8	12[a]	16[a]
		Soil NO_3^--N (35 cm)[a]		
Control	210.2 (3.8)[b]	233.7 (2.1)	269.5 (0.9)	250.1 (3.4)
Planted	91.3 (7.5)	3.5 (0.5)	5.5 (1.2)	8.4 (1.1)
		Soil NH_4^+-N (35 cm)[a]		
Control	6.7 (0.2)	1.4 (0.1)	4.3 (0.2)	6.5 (0.1)
Planted	0.3 (0.1)	1.6 (0.2)	2.6 (0.3)	1.2 (0.2)
		Soil $^{15}NO_3^-$-N (excess, 35 cm)[a]		
Control	22.8 (0.7)	24.4 (3.2)	34.7 (1.5)	30.1 (1.9)
Planted	0.7 (0.3)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
		Soil $^{15}NH_4^+$-N (excess, $g\ ha^{-1}$, 35 cm)[a]		
Control	28 (10)	1 (1)	6 (0)	5 (0)
Planted	4 (1)	2 (2)	13 (7)	2 (2)

The proportion of biomass N in soil total N was similar between control and planted soil and varied from 1.7 to 2.1 (average, 1.9%) and from 1.9 to 2.1 (average, 2%), respectively (Table 4). However, the proportion of biomass ^{15}N in soil total ^{15}N varied greatly with plant growth and between control and planted soils. Twenty-one to 27% (average, 24%) of soil ^{15}N was found in microbial biomass in the control soil and from 11 to 21% (average, 16%) in the planted soil. Approximately a 10-fold greater proportion of biomass ^{15}N in total soil ^{15}N than that of blomass N in soil total N suggested that the inorganic fertilizer ^{15}N was more accessible for microbial synthesis than soil-native N. The ratio of biomass ^{15}N to soil ^{15}N is an indicator of net immobilization and mineralization. The change of ratios of biomass ^{15}N to soil ^{15}N with time in the planted soil tended to oscillate (Table 4), which is similar to the findings of White *et al.* (1988) who showed that N-dynamics demonstrated one or more oscillations between net mineralization and net immobilization after addition of C (as a sucrose–sawdust mixture). Unlike the oscillation in biomass ^{15}N, the ratio of total biomass N to soil N remained fairly constant (Table 4) which agrees with conclusions of Kelley and Stevenson (1987) that newly immobilized N constitutes a relatively labile pool of organic N, which is more susceptible to transformation than native organic N.

Soil NO_3^-- and $^{15}NO_3^-$-N concentrations in the control soil demonstrated similar dynamic patterns, which were directly associated with N application (Table 5). The soil NO_3^-- and $^{15}NO_3^-$-N increased until the third harvest in proportion to the increasing N fertilization at each harvest of 40, 60, and 100 kg N ha^{-1}, and decreased somewhat from the third to fourth harvest because of the longer time and greater opportunity for N gaseous loss from soil (Table 6). Soil NO_3^-- and $^{15}NO_3^-$-N of planted soil decreased significantly from the first to second harvest because of the rapid uptake of N with plant growth and increasing immobilization of N by microbial biomass (Table 4), although 50% more N (20 kg N ha^{-1}) was applied after the first harvest. After the second harvest, soil NO_3^-- and $^{15}NO_3^-$-N contents remained low, despite the greater amount of total ^{15}N applied. Compared to soil NO_3^-- and $^{15}NO_3^-$-N, the soil NH_4^+- and $^{15}NH_4^+$-N concentrations were negligible at various harvest times in both control and planted soils, indicating a predominance of nitrification in these soils which removed mineralization products (NH_4^+) before they accumulated (Table 5).

Of ^{15}N applied to soil, 16–31% remained in planted soil and 80–107% in control soil at various harvest times (Table 3). Of the ^{15}N remaining in soil, only 0.5–5.4% (0.1–0.7 kg N ha^{-1}) was in the form of inorganic ^{15}N in planted soil in contrast to the control soil in which 52–71% (23–69 kg N ha^{-1}) remained as inorganic N. Of the ^{15}N remaining in cropped and bare soil as previously discussed, an average of 16 and 24% (2 and 10 kg N ha^{-1}) was found in microbial biomass which left 82 and 13% (10 and 6 kg N ha^{-1}) as non-biomass organic N, respectively. This suggests that the cycling of N through microbial biomass was more rapid in planted than in non-planted soil. Plant roots apparently released C substrates which promoted the immobilization of inorganic N. In other words, N cycling in soil was enhanced by root-derived C.

Denitrification

Maximum rates of denitrification, gaseous ^{15}N loss, N_2O flux, and soil respiration from control and planted soils are presented in Figs 2, 3, 4 and 5. Denitrification and N_2O losses from planted soil were low throughout the growth period, ranging from 1.2 to 136 g N ha^{-1} d^{-1}, when soils were generally drier and less than 50% of the soil pore space was water-filled before watering. When soils were wetted by irrigation to 85–95%, denitrification losses ranged from 0.02 to 3.4 kg N ha^{-1} d^{-1} with N_2 comprising 70–99% of denitrification gases measured. Gaseous ^{15}N losses, including aerobic

Table 6. Cumulative loss of denitrification, gaseous ^{15}N, and nitrous oxide from control and planted soils at various harvest times

Components	Growth period (weeks after emergence)			
	4	8	12	16
		Cumulative losses (kg N ha^{-1})		
		Control soil		
Denitrification	5.0	14.5	40.3	51.6
N_2O emission	1.9	5.4	7.7	9.5
Gaseous ^{15}N	2.3	6.6	18.0	22.2
		Planted soil		
Denitrification	11.5	17.9	30.6	39.9
N_2O emission	2.2	4.1	5.7	6.4
Gaseous ^{15}N	3.2	7.8	13.8	15.6
		Gaseous ^{15}N loss as a percentage of ^{15}N applied		
Control soil	5.9	11.1	18.2	22.5
Planted soil	8.1	13.2	14.0	15.9
		Ratio of N_2O-N to (N_2O + N_2)-N		
Control soil	0.38	0.37	0.19	0.18
Planted soil	0.19	0.23	0.19	0.16

and anaerobic ^{15}N gas production, were similar to the trends observed for denitrification and N_2O and ranged from 1 to 41 g N ha^{-1} d^{-1} before watering and from 0.2 to 669 g N ha^{-1} d^{-1} after watering. Rates of denitrification, gaseous ^{15}N loss, and N_2O emission between control and planted soils differed significantly in most cases, apparently due to differences in soil water content, amounts of NO_3^- and NH_4^+, and soil available C, resulting from the presence of maize plants. The maximum denitrification and gaseous ^{15}N loss rates observed appeared closely related to soil NO_3^- content and available C as discussed earlier for these stages of growth (Figs 2 and 3). The maximum denitrification and gaseous ^{15}N loss in planted soil were significantly ($P < 0.05$) greater than those in non-planted control soil at the first and second gas sampling times during the early growth of maize, when soil NO_3^--N in both control and planted soils exceeded 8–12 kg ha^{-1} (2–3 mg kg^{-1}) and available C from plant roots was sufficient in planted soil (Tables 5 and 2). However, the denitrification and gaseous ^{15}N losses from control soil were significantly greater than those from planted soil after the third gas sampling time. This apparently resulted from limiting amounts of NO_3^- levels for denitrification in planted soil (Table 5), even though sufficient C was available to denitrifying bacteria as indicated by relatively high soil respiration rates.

The maximum N_2O emission rates and soil respiration showed a different pattern from the maximum denitrification rates and gaseous ^{15}N losses (Figs 4 and 5). The maximum N_2O emission from planted soil was equal to that from non-planted control soil for the first two sampling times but was significantly lower throughout the rest of the growth period. However, the maximum CO_2 flux of planted soil was significantly greater than that of the non-planted control soil throughout the growth period and apparently resulted from plant root respiration and additional microbial respiration of root exudates in excess of microbial respiration of soil organic C as indicated by the control.

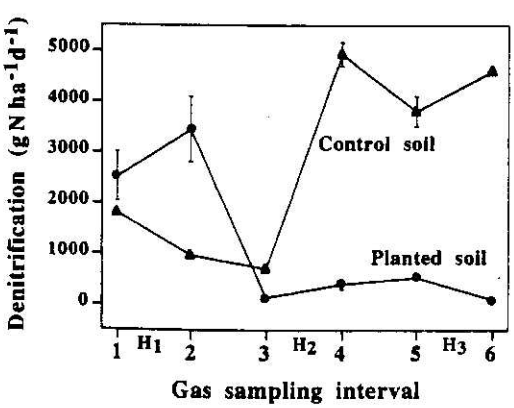

Fig. 2. Maximum denitrification rates of control and planted soils at different sampling times. Harvest times indicated by H_1–H_3. Vertical bars show standard errors.

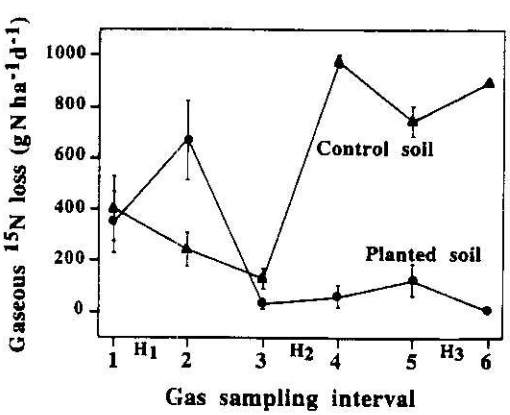

Fig. 3. Maximum gaseous ^{15}N loss rates of control and planted soils at different sampling times. Harvest times indicated by H_1–H_3. Vertical bars show standard errors.

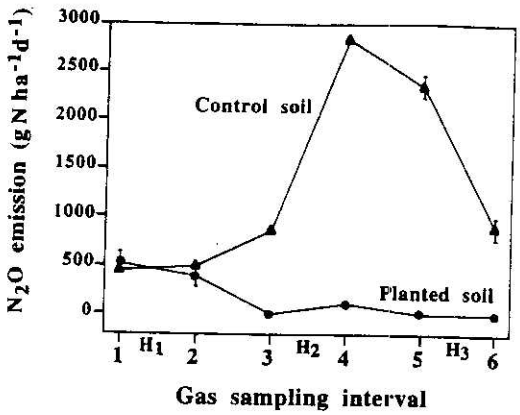

Fig. 4. Maximum N_2O emission of control and planted soils at different sampling times. Harvest times indicated by H_1–H_3. Vertical bars show standard errors.

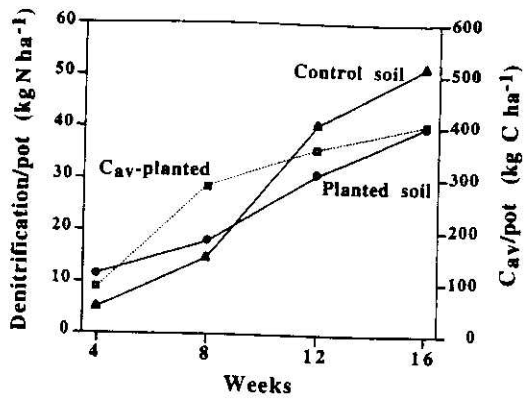

Fig. 6. Cumulative denitrification losses and root-derived available C (C_{av}) during growth of maize in the greenhouse.

The estimated cumulative N losses via denitrification and N_2O emission at different growth periods are shown in Table 6. The cumulative N losses as denitrification, gaseous ^{15}N, and N_2O emission increased with time in both control and planted soils. The cumulative gaseous ^{15}N loss at different growth stages ranged from 2 to 22 kg N ha^{-1} in control soil, which accounted for 6–23% of the ^{15}N applied to soil, and from 3 to 16 kg N ha^{-1} in the planted soil that represented 8–16% of the ^{15}N applied to soil (Table 6). Similar to the maximum denitrification rates of control and planted soils at different gas sampling times, the cumulative losses via denitrification from planted soil at the first and second harvest were significantly ($P < 0.05$) greater than those from control soil, and then became significantly lower at the third and fourth harvest (Fig. 6). This apparently occurred because the soil NO_3^- concentration became a limiting factor for denitrification in planted soil after the second harvest (Table 5). Previous research also suggested that enhancement of denitrification near plant roots occurred only at a relatively high NO_3^- concentration (10 μg g^{-1}) in forest soils ranging in texture from sandy loam to loamy sand (Smith and Tiedje, 1979).

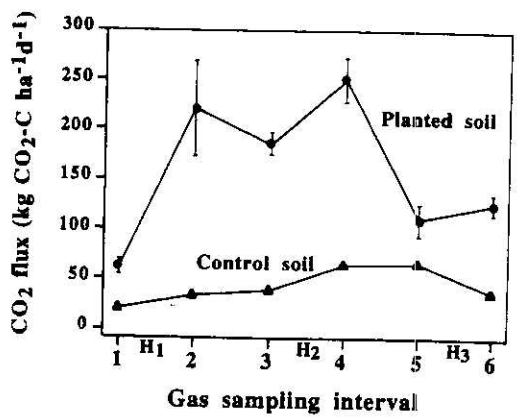

Fig. 5. Maximum CO_2 flux of control and planted soils at different sampling times. Harvest times indicated by H_1–H_3. Vertical bars show standard errors.

The cumulative losses via denitrification from planted soil at 4 and 8 wk were 2.30- and 1.23-fold greater, respectively, than those from control soil (Fig. 6), which apparently resulted from available C differences in the rhizosphere, i.e. planted soil received additional available C sources from the plant roots with an average of 186 kg C ha^{-1} during the first 8 wk, as shown by the dotted line in Fig. 6. This also showed that the root-derived available C contributed 19–57% (average of 29%) of total denitrification in cropped soil. In other words, the presence of plants increased denitrification losses from soil by an average of 29% during early growth stages. According to the stoichiometric relationship of microbial reduction of NO_3^- to N_2O or N_2, 1 kg of available C is required for production of 1.17 kg N as N_2O or 0.93 kg N as N_2 (Burford and Bremner, 1975). Calculations based on these values indicate that 1 and 1.03 kg of available C are required in control and planted soil, respectively, to produce 1 kg of N as $N_2O + N_2$ in which the ratio of N_2O-N to ($N_2O + N_2$)-N is the average of ratios for control soil (0.28) and planted soil (0.19), respectively (Table 6), If we assume that the C used by denitrification in planted soil was from root exudates only, the C consumed for denitrification accounted for only 6–13% (average, 10%) of root-derived available C (Fig. 6). The balance of 40% was incorporated into soil microbial biomass, and 50% was respired as non-nitrate-respiration, which suggested that the root-derived available C far exceeded the amount needed for denitrification. It is expected that root-derived available C would have a significantly greater contribution to denitrification

in soils with deficient available C and adequate NO_3^- content. In our study, the amounts of mineralizable C observed from laboratory incubation of disturbed soil were large enough to support the denitrification rates measured in the greenhouse (Table 2).

Soil respiration rates ranged from 8.4 to 65 and 22.4 to 250 kg CO_2-C ha^{-1} d^{-1} in control and planted soil, respectively, and varied with soil water status and growth periods (Fig. 5). Based on the measured denitrification rates, the CO_2 produced from denitrification ranged from 0.05 to 4.9 and 0.01 to 3.4 kg CO_2-C ha^{-1} d^{-1}, which account for only 0.6–7.5 and 0.1–1.4% of total soil respiration measured from control and planted soil, respectively. Respiration rates of these magnitudes provide additional evidence that soil-available C contents were adequate for microbial denitrification to occur in the presence or absence of plants and also may contribute appreciably to denitrification in spite of drier soil conditions. High respiration rates significantly reduce O_2 supply in soil, which can stimulate denitrification. Previous results showed that CO_2 production rates ranging from 166 to 240 kg C ha^{-1} d^{-1} could explain 31–47% of the variation in denitrification when soil was fairly dry with WFPS less than 60% (Rice *et al.*, 1988).

CONCLUSIONS

Our findings confirmed the hypothesis that root-derived available C enhances microbial transformations of N in soil, specifically immobilization-mineralization and gaseous N loss via microbial denitrification. At maize maturity, 15% of the soil microbial biomass was derived from root-released C sources, which amounted to 402 kg C ha^{-1} and represented about one-third of the total C released from roots during plant growth. Nitrogen transformations in the root zone were dominated by C dynamics and the release of available C from maize roots. In the presence of plants, 67% more soil mineral N was immobilized in organic N than in unplanted soil, despite a higher competition by plants for mineral N uptake. Maximum microbial denitrification rate was 1.5 times greater, and cumulative denitrification losses 77% greater when adequate NO_3^- was present in soil, and root-derived available C increased microbial denitrification by 19–57% during early growth of maize. Therefore, we conclude that the presence of plants enhances microbial transformations of N in the rhizosphere, specifically immobilization and gaseous N loss via denitrification.

An assumption in using the $\delta^{13}C$ change in microbial biomass to estimate root-derived available C, is that there is no isotope fractionation during microbial metabolism of ^{13}C enriched C_4 plant matter. In our study, the $\delta^{13}C$ signature of soil respiration from unplanted soils indicated that preferential utilization of ^{12}C, or fractionation, from native organic matter had occurred, but this finding was not supported by other data and was assumed to be an artifact of experimental method. However, if isotopic fractionation of plant derived C occurred in our study, the values obtained for root-derived available C are underestimated, and our conclusions regarding the importance of plant derived C are strengthened.

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